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EFFECTS OF SODIUM PENTOBARBITAL ANESTHESIA AND LEUKOCYTOSIS ON --ETC(U)

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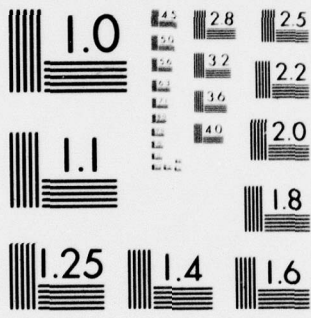
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TECHNICAL REPORT NO. 129

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E. COLI MORTALITY IN DOG BLOOD IN VITRO

Linda T. Archer, Gary L. White, Beverly K. Beller,  
Ora F. Elmore, Jeanette S. Glasgow, and Lerner B. Hinshaw

Prepared for Publication

in

Proceedings of the Society of  
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EFFECTS OF SODIUM PENTOBARBITAL ANESTHESIA AND LEUKOCYTOSIS ON

'Escherichia coli' MORTALITY IN DOG BLOOD 'IN VITRO'

Linda T. Archer, Gary L. White, Beverly K. Beller,  
Ora F. Elmore, Jeanette S. Glasgow, and Lerner B. Hinshaw

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*Introduction.* Pathologic manifestations of live E. coli organism shock in the canine species include progressively developing hypoglycemia, systemic hypotension and liver dysfunction (1,2). Recent reports from this laboratory have linked survival of the dog in shock to leukocytosis, normoglycemia and sustained gluconeogenic function (3-6). Sublethal intravenous injections of E. coli endotoxin in the awake dog produce marked leukocytosis (3-6) and subsequent survival of  $2 \times LD_{100}$  injections of either E. coli organisms or endotoxin (3-5). These studies reveal accelerated glucose utilization in the leukocytotic blood compared with normocytotic blood following incubation with live E. coli organisms in vitro (4). The neutrophil's phagocytic activity has been implicated as the primary factor accounting for this elevated glucose uptake (4).

Circulating neutrophils play a key role in host defense against bacterial organisms in septic shock (7-9). Neutrophil dysfunction can adversely affect recovery from bacterial infection and shock (10,11), and increased concentrations of neutrophils are apparently critical in reducing mortality (4,5). Intravenous pentobarbital anesthesia causes a significant decrease in leukocyte concentration in dogs (12), and various barbiturates have cytotoxic effects on tissue-cultures of rat lymphocytes (13). General anesthesia in man inhibits phagocytosis by neutrophils in vitro (14), while halothane anesthesia increases mortality in mice subjected to fecal peritonitis (15). Since investigations employing anesthesia during septic shock may have compromised host-defense, the present study was designed to determine if sodium pentobarbital anesthesia depresses glucose metabolism of leukocytes,

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affects survival of neutrophils or modifies the survival rate of live E. coli organisms added to blood in vitro.

*Methods.* Twelve awake adult mongrel dogs of either sex were used for these studies. Dogs were selected for freedom of clinical signs of disease, absence of microfilaria or heartworms, treated for intestinal parasites, and stabilized for 3-6 weeks in our animal research facility.

Animals were divided into two groups of six dogs each. Group B, experimental dogs, received E. coli endotoxin (Difco; Detroit, Mich.), 0.003 mg/kg on Days 1 and 2, and 2.25 mg/kg ( $LD_{100}$ ) on Day 3. Group A, control animals, received equal volumes of saline on Days 1, 2 and 3. On Day 4, blood for the in vitro studies was drawn intravenously into plastic syringes wet with heparin (1000 U/ml concentration) from awake dogs in Groups A and B. The dogs in Group B were then anesthetized with sodium pentobarbital (28-30 mg/kg) and allowed to stabilize 30 minutes, at which time a second blood sample was collected.

Immediately following each collection, blood was divided into 10 ml volumes in separate sterile plastic tubes, each containing 0.1 ml heparin (20,000 U/ml), capped, gently mixed and placed on ice. The blood from Group A dogs (saline-pretreated) was separated into two 10-ml aliquots and designated as Tubes A1 and A2 while blood from Group B dogs (endotoxin-pretreated) was separated into two 10-ml volumes before anesthesia and designated Tubes B3 and B4, and after anesthesia, B5 and B6 (see Figure 1). Live E. coli organisms were prepared as previously reported (16,17) and a  $LD_{100}$ , 0.25 ml ( $8.0 \times 10^9$

organisms/ml concentration), was added to Tubes A2, B4 and B6 while equal volumes of saline were added to Tubes A1, B3 and B5. The 10-ml aliquots were separated into 5-ml volumes and one set of tubes, A1 through B6, was simultaneously placed in a 37-38°C water bath, incubated and gently tumbled for the duration of the experiments. In vitro blood glucose concentrations were determined hourly with a Beckman glucose analyzer (Beckman Instruments; Fullerton, Calif.) possessing an accuracy of  $\pm 3$  mg%, and each experiment was terminated when blood glucose fell to an average concentration of 12 mg%. The extra set of tubes (A1 through B6) was sampled initially for E. coli viability counts, glucose concentration, total leukocyte and differential leukocyte concentrations, and discarded. In all three tubes containing E. coli, glucose reached a mean of 12 mg% in an average of +2 hours of incubation and at that time the final sample for total leukocyte, differential leukocyte and E. coli concentrations was drawn. Initial and final white blood cell (WBC) counts were measured with an automatic particle counter (Coulter Z<sub>F</sub>; Hialeah, Fla.), the WBC differential by microscopic examination of blood smears stained with Wright's stain, and E. coli viability using standard serial dilution and pour-plate techniques.

The LD<sub>100</sub>,  $1.25 \times 10^{10}$  organisms/kg, determined in the earlier in vivo studies (4), was calculated for use in the present in vitro studies by assuming an in vivo blood volume of 90 ml/kg and instantaneous mixing of organisms in vivo, and the in vitro organism concentration thus selected was  $1.4-1.5 \times 10^8$  organisms/ml blood.

The leukocytotic blood from each experimental dog (endotoxin-pretreated), after the separate additions of either live E. coli organisms (LD<sub>100</sub>) or



saline in vitro, was used to test the effect of sodium pentobarbital anesthesia on glucose uptake, total leukocyte concentration, differential leukocyte concentration and E. coli viability.

Results from all experiments were analyzed using the "t" test for paired or unpaired data.

*Results.* Table I shows the effect of sodium pentobarbital anesthesia and live E. coli organisms on total leukocyte concentration. Sublethal injections of E. coli endotoxin for three consecutive days caused a significant leukocytosis ( $28,100/\text{mm}^3$ ) in Group B dogs compared with the  $10,300/\text{mm}^3$  leukocyte count in the saline-pretreated animals (Group A). When the blood from Tubes B3 and B5 are compared with that from Tubes B4 and B6, it can be seen that sodium pentobarbital reduced ( $p < 0.01$ ) the circulating leukocyte concentration. Although the addition of live organisms in vitro caused a decrease ( $p < 0.005$ ) in total leukocytes (Tubes A2, B4 and B6), the mean reduction before anesthesia ( $-8800$  in Tube B4) was not statistically different from the mean decrease after anesthesia ( $-10,500$  in Tube B6).

The effect of pentobarbital anesthesia and E. coli organisms on neutrophil survival in vitro are shown in Tables IA, IB and IC. Differential leukocyte counts show that the leukocytosis was primarily due to an elevated mature neutrophil count ( $24,100/\text{mm}^3$ ) although immature neutrophils were also increased ( $p < 0.05$ ) when compared with saline controls. As a function of the decrease in total circulating leukocytes after anesthesia (see Table I), mature neutrophil concentration is significantly reduced ( $p < 0.02$ ) Tubes B3 and B4,  $24,100$  and  $24,000, \#/\text{mm}^3$ , compared with Tubes B5 and B6,  $20,700$  and  $20,900, \#/\text{mm}^3$ )



(Table IA). Results show that sodium pentobarbital anesthesia does not alter the survival rate of mature or immature neutrophils during the mean 2-hour in vitro exposure time. Addition of E. coli organisms in a  $LD_{100}$  concentration markedly decreases the numbers of mature neutrophils ( $p < 0.02$ ) and elevates the numbers of degenerated neutrophils ( $p < 0.02$ ). Although the loss of mature neutrophils is significantly greater ( $p < 0.05$ ), -7500 in Tube B4 and -8700 in Tube B6 in endotoxin-pretreated blood compared with saline-pretreated blood (-2800 in Tube A2), there is no difference in neutrophil loss during the mean in vitro exposure time of 2 hours in blood drawn before or after sodium pentobarbital injection. There were no significant changes in monocyte or lymphocyte concentrations in tubes containing live E. coli organisms whether blood was saline-pretreated (normocytotic) or endotoxin-pretreated (leukocytotic) before or after anesthesia.

Figure 2 illustrates the effects of in vitro live E. coli organisms ( $LD_{100}$ ) and sodium pentobarbital anesthesia on mean cumulative glucose uptake in blood from three paired experiments (total,  $N=36$ ; i.e., 3 sets of 12 experiments each). The saline-pretreated dog blood (Tubes A1 and A2) was obtained from six animals and either  $LD_{100}$  live E. coli organisms or saline was added to separate tubes in vitro immediately before zero time. The endotoxin-pretreated dog blood was obtained from six animals before anesthesia (Tubes B3 and B4), from the same six dogs after anesthesia (Tubes B5 and B6), and again either  $LD_{100}$  live E. coli organisms or saline was added. The addition of live organisms (upper three curves) results in a markedly elevated glucose uptake during all time periods ( $p < 0.05$ ) compared with each paired saline control tube. The administration of sodium pentobarbital in vivo did not change the rate of glucose uptake when tubes containing saline

(B3-B5) or containing E. coli (B4-B6) were compared. Glucose uptake was elevated ( $p < 0.01$ ) in the endotoxin-pretreated leukocytotic blood in the presence of live organisms (Tubes B4 and B6) compared with saline-pretreated normocytotic blood with E. coli added (Tube A2).

Table II depicts the effects of in vivo anesthesia on E. coli mortality in vitro. In the neutrophilic blood from endotoxin-pretreated dogs, the number of live E. coli organisms both before and after sodium pentobarbital anesthesia was significantly reduced ( $p < 0.01$ ), from  $1.5 \times 10^8$  to  $1.8 \times 10^6$  before anesthesia and from  $1.4 \times 10^8$  to  $1.6 \times 10^6$  after anesthesia, compared with the reduction of  $1.5 \times 10^8$  to  $1.4 \times 10^7$  organisms/ml in the blood with fewer neutrophils from saline-pretreated animals. The E. coli mortality rate in vitro for the 2-hour incubation period was 99% before and after anesthesia for neutrophilic blood, which was a greater reduction ( $p < 0.005$ ) than in the saline-pretreated (no anesthesia) blood of 91%. The percent reduction in numbers of E. coli in all three tubes underscores the neutrophil's great capacity for bacterial phagocytosis.

Measurement of the in vitro red blood cell (RBC) concentrations documented the stability of the in vitro system for the 2-hour incubation period; initial and final RBC concentrations were not significantly altered in any of the six tubes (A1, A2, B3-B6).

*Discussion. Role of neutrophils in shock.* Neutrophils perform a key role in the clearance of bacterial organisms in septic shock (7-9) and their dysfunction can adversely influence the course of bacterial infections (10,11). Apparently greater numbers of neutrophils improve survival rate in septic shock (4,5), but the adequate provision of host-defense may exact an increased metabolic

cost (4,5,16). Since intravenous sodium pentobarbital anesthesia in dogs causes a significant reduction in circulating leukocytes and granulocytes (12) and general anesthesia in man inhibits phagocytosis of neutrophils in vitro (14), the present study was designed to evaluate the effect of sodium pentobarbital anesthesia on glucose metabolism of leukocytes, survival rates of neutrophils and mortality rate of live E. coli organisms in vitro.

*Effects of anesthesia on survival rate of leukocytes and neutrophils.*

In agreement with previous reports from this laboratory, intravenous administration of daily sublethal doses of E. coli endotoxin caused a marked leukocytosis accounted for mainly by an increase in mature and immature neutrophils (3-6). Although in vivo administration of sodium pentobarbital causes a reduction in leukocyte concentration, as previously reported (12), the incubation of blood in vitro from both anesthetized or unanesthetized dogs in the absence of E. coli organisms does not affect the survival rate of leukocytes. Addition of live E. coli to blood markedly decreases the concentration of leukocytes, but the leukocyte loss was not significantly different before or after anesthesia.

Mature neutrophils accounted for 87% of the total leukocyte count in the leukocytotic endotoxin-pretreated blood in contrast to 57% in normocytotic blood. Numbers of mature neutrophils in all tubes were significantly reduced in the presence of E. coli, but this loss in vitro was similar before and after anesthesia. A significant increase in degenerated neutrophils was seen in the presence of E. coli which was uninfluenced by anesthesia. Baboon blood subjected to live E. coli also demonstrated significant decreases in mature neutrophils and increases in degenerated neutrophils (17).

*Effect of anesthesia on metabolic activity of neutrophils.* Previous studies have suggested that increased numbers of neutrophils protect animals from  $2 \times LD_{100}$  E. coli endotoxin (3,6) or live E. coli organisms (4,5), although they may use more substrate in the process (3-6,16). Glucose uptake was also markedly elevated in the present study after addition of E. coli in the leukocytotic (neutrophilic) blood compared with the normocytotic blood (4). Findings show, however, that pentobarbital anesthesia did not alter glucose uptake of blood in the presence or absence of live E. coli organisms.

*Effect of anesthesia on E. coli survival rate.* E. coli mortality was significantly increased in both endotoxin-pretreated or saline-pretreated dog blood, although it was higher in the presence of increased numbers of neutrophils. Sodium pentobarbital anesthesia did not alter E. coli mortality rate in the leukocytotic blood.

*Effect of anesthesia and leukocytosis in vitro.* Although findings in the present study reveal reductions in leukocyte and neutrophil concentrations after anesthesia, these decreases did not alter glucose uptake, neutrophil loss in the in vitro blood system or E. coli mortality. The accelerated glucose uptake, mature neutrophil loss and live E. coli mortality were greater in the neutrophilic blood with E. coli added, suggesting higher rates of phagocytosis.

*Summary.* Data reveal that sodium pentobarbital anesthesia does not depress glucose uptake of neutrophils stimulated by the addition of live E. coli. E. coli viability was reduced equally in blood obtained before and after sodium pentobarbital anesthesia. Pre- and post-anesthetic blood samples with E. coli added showed comparable decreases in mature neutrophils and elevations in numbers of degenerated neutrophils. These data suggest that the ability of the neutrophil to phagocytize is not affected by sodium pentobarbital anesthesia.

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TABLE I. EFFECTS OF SODIUM PENTOBARBITAL ANESTHESIA AND LD<sub>100</sub> LIVE E. COLI ORGANISMS  
ON TOTAL LEUKOCYTE CONCENTRATION IN DOG BLOOD IN VITRO (N=6)

Group	Test tube designation <sup>a</sup>	*	Initial mean concentration; (#/mm <sup>3</sup> ); zero time	§	Final mean concentration; (#/mm <sup>3</sup> ); +2 hours	Mean $\Delta$ change	†
Saline-pretreated; no anesthesia	A1		10100	NS	10400	+300	
	A2		10400	.005	7500	-2900	.01
Endotoxin-pretreated; pre-anesthesia	B3		28100	NS	27700	-370	
	B4		28100	.005	19300	-8800	.005
Endotoxin-pretreated; post-anesthesia	B5	.005	24600	NS	24500	-150	
	B6	.01	24700	.001	14300	-10500	.001
Unpaired comparison:							
A1 - B3			.001			NS	
A1 - B5			.005			NS	
A2 - B4			.001			.005	
A2 - B6			.005			.001	

<sup>a</sup>Test tubes designated A1, B3, B5 contain blood with saline only (no E. coli). Test tubes designated A2, B4, B6 contain blood with saline and E. coli.

\*Paired comparison; B3 - B5, B4 - B6, initial to initial concentrations.

§Paired comparison; initial to final concentrations.

†Paired comparison; A1 - A2, B3 - B4, B5 - B6, mean  $\Delta$  change.

TABLE IA. EFFECTS OF SODIUM PENTOBARBITAL ANESTHESIA AND LD<sub>100</sub> LIVE E. COLI ORGANISMS  
ON MATURE NEUTROPHIL CONCENTRATION IN DOG BLOOD IN VITRO (N=6)

Group	Test tube designation <sup>a</sup>	*	Initial mean concentration; (#/mm <sup>3</sup> ); zero time	§	Final mean concentration; (#/mm <sup>3</sup> ); zero time	Mean $\Delta$ change	†
Saline-pretreated; no anesthesia	A1		5700	.02	5000	-700	NS
	A2		5800	.01	3100	-2800	
Endotoxin-pretreated; pre-anesthesia	B3		24100	.05	22300	-1800	.025
	B4		24000	.02	16600	-7500	
Endotoxin-pretreated; post-anesthesia	B5	.005	20700	NS	21100	+400	.005
	B6	.02	20900	.005	12100	-8700	
Unpaired comparison:							
A1 - B3			.001			NS	
A1 - B5			.001			.05	
A2 - B4			.001			.05	
A2 - B6			.001			.01	

See legend to Table I.

TABLE 1R. EFFECTS OF SODIUM PENTOBARBITAL ANESTHESIA AND LD<sub>100</sub> LIVE *E. COLI* ORGANISMS  
ON IMMATURE NEUTROPHIL CONCENTRATION IN DOG BLOOD IN VITRO (N=6)

Group	Test tube <sup>a</sup> designation	*	Initial mean concentration; (#/mm <sup>3</sup> ); zero time	§	Final mean concentration; (#/mm <sup>3</sup> ); +2 hours	Mean Δ change	†
Saline- pretreated; no anesthesia	A1		340	NS	579	+239	NS
	A2		235	NS	150	-86	
Endotoxin- pretreated; pre-anesthesia	B3		1646	NS	2823	+1176	.05
	B4		1377	NS	45	-1332	
Endotoxin- pretreated; post-anesthesia	B5	NS	1814	NS	1312	-502	NS
	B6	NS	1523	NS	114	-1409	
Unpaired comparison:							
A1 - B3			.05			NS	
A1 - B5			.025			.025	
A2 - B4			NS			NS	
A2 - B6			NS			NS	

See legend to Table I.



TABLE IC. EFFECTS OF SODIUM PENTOBARBITAL ANESTHESIA AND LD<sub>100</sub> LIVE *E. COLI* ORGANISMS ON DEGENERATED NEUTROPHIL CONCENTRATION IN DOG BLOOD IN VITRO (N=6)

Group	Test tube designation <sup>a</sup>	*	Initial mean concentration; (#/mm <sup>3</sup> ); zero time	§	Final mean concentration; (#/mm <sup>3</sup> ); +2 hours	Mean $\Delta$ change	†
Saline-pretreated; no anesthesia	A1		0	NS	0	0	
	A2		0	.005	1097	+1097	.005
Endotoxin-pretreated; pre-anesthesia	B3		0	NS	0	0	
	B4		43	.01	1122	+1079	.01
Endotoxin-pretreated; post-anesthesia	B5	NS	0	NS	0	0	
	B6	NS	20	.02	1204	+1184	.02
Unpaired comparisons:							
A1 - B3			NS			NS	
A1 - B5			NS			NS	
A2 - B4			NS			NS	
A2 - B6			NS			NS	

See legend to Table I.



TABLE II. EFFECTS OF SODIUM PENTOTHAL ANESTHESIA ON E. COLI MORTALITY

## IN DOG BLOOD IN VITRO (MEAN±SE)

Group	Test tube <sup>a</sup> designation	Initial mean concentration; (# <u>E. coli</u> /ml); zero time	*	Final mean concentration; (# <u>E. coli</u> /ml); +2 hours	% change
Saline- pretreated; no anesthesia	A2	1.5x10 <sup>8</sup> (1.4x10 <sup>7</sup> )	.001	1.4x10 <sup>7</sup> (3.5x10 <sup>6</sup> )	-91 (2.2)
Endotoxin- pretreated; pre-anesthesia	B4	1.5x10 <sup>8</sup> (1.0x10 <sup>7</sup> )	.001	1.8x10 <sup>6</sup> (3.0x10 <sup>5</sup> )	-99 (0.2)
Endotoxin- pretreated; post-anesthesia	B6	1.4x10 <sup>8</sup> (2.0x10 <sup>7</sup> )	.001	1.6x10 <sup>6</sup> (2.0x10 <sup>5</sup> )	-99 (0.2)
Unpaired comparisons:					
A2 - B4		NS		.01	.005
A2 - B6		NS		.01	.005

<sup>a</sup>Test tubes designated A2, B4, B6 contain blood with saline and E. coli.

\*Paired comparison; initial to final concentrations

#### FIGURE LEGENDS

- Figure 1. Illustrates the pretreatments of blood donor dogs and treatments of blood samples for study in vitro. Group B, experimental dogs, received E. coli endotoxin, 0.003 mg/kg, on Days 1 and 2, and 2.25 mg/kg ( $LD_{100}$ ) on Day 3. Group A, control animals, received equal volumes of saline on Days 1, 2 and 3. On Day 4, blood for in vitro studies was drawn from unanesthetized dogs in Groups A and B. Then dogs in Group B were anesthetized with sodium pentobarbital (28-30 mg/kg) and allowed to stabilize 30 minutes, at which time a second blood sample was collected.
- Figure 2. Effect of sodium pentobarbital anesthesia and E. coli organisms on cumulative glucose utilization in whole blood in vitro following previous sublethal injections of E. coli endotoxin in vivo (N=6 in each group; total N = 36). E. coli or saline was added to each test tube immediately before zero time;  $1.5 \times 10^8$  organisms/ml of blood. Blood was drawn from saline-pretreated dogs with no anesthesia and from endotoxin-pretreated dogs before and after anesthesia (see Fig. 1 for details). Glucose concentrations (mean $\pm$ SE) are plotted and symbols ( $\circ$ ,  $\Delta$ ,  $\square$ ) located next to P values show paired statistical comparisons of Tubes A1 to A2, B3 to B4, and B5 to B6. The symbol ( $\circ$ ) located next to P values indicate unpaired statistical comparison of Tubes A2 to B4 and B6.

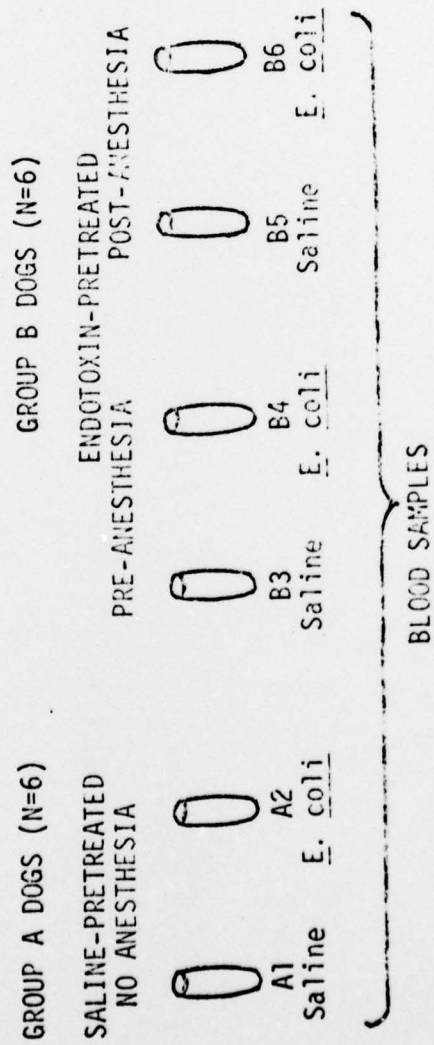


Fig. 1

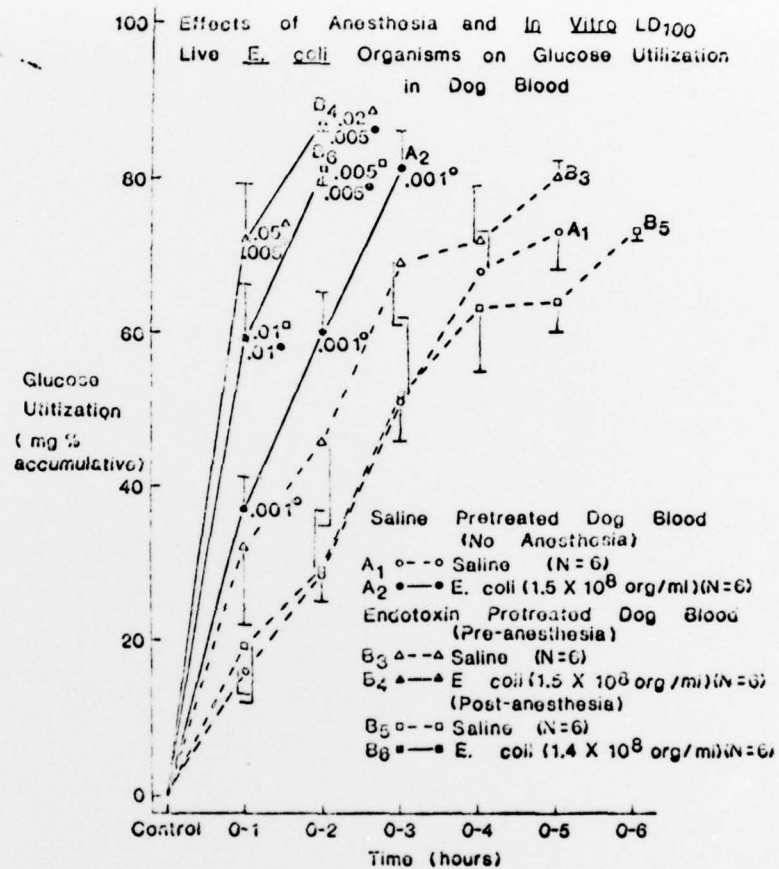


Fig 2



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